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A Potential Alkaloid Compound Isolated from A Marine Sponge Collection Number MD-02 Againts Cancer

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Abstract

Majority of the currently available anticancer drugs are designed to have selective toxicity to rapidly dividing cells. Among these agents the focus of many studies are compounds obtained from natural products with high therapeutic index. In this study the cytotoxicity of a marine alkaloid compound isolated from sponges collection number MD-02 on cancer and normal cells was evaluated.
The compound was obtained from chloroform fractions of sponges followed by Vacuum Liquid Chromatography and TLC-preparative. This compound in a concentration dependent manner inhibit the growth of human cancer cells and were more toxic to Raji than HeLa cells with LC_{50} of 6.37 and 22.67 µg/mL, respectively. Cells treated with this compound showed cleavage of chromosomal DNA into fragments, suggesting the possibility of apoptotic cell death. This compound was less toxic to normal Vero cells showing its selectivity with LC_{50} of 100.36 µg/mL.

Key Words: Antitumor agents, sponges, alkaloid, HeLa, Raji, Vero

Intoduction

Until recently, cancer is still a problem and common cause of death around the world. Various therapeutic modalities have been employed in the fight against cancer. These include: alkylating agents, anti-metabolites, radiomimetic drugs, hormones and antagonists, surgery and miscellaneous agents (Cram *et al.*, 1992; Calabresi and Chabner, 1991; Hoppe *et al.*, 1992; Lorgan *et al.*, 1996; Borgstrom *et al.*, 1982). However non of these agents has produced satisfactory anticancer effect without relapse and most time, their therapeutic activity is accompanied by debilitating side effects (Green *et al.*, 1982; Herzig *et al.*, 1987). Despite the introduction of new drugs for the treatment of cancer, the overall survival of patients suffering from this malignancy is far from satisfactory.

A number of researches have been conducted to search anticancer with renewed vigor. Natural products are the major source of lead compounds for drugs against cancer. Marine invertebrates are known as rich sources of compounds with unique chemical structures and pronounced chemical activities, which suggests potential value as lead structures for the development of new pharmaceuticals.

A diverse range of bioactivities of these natural resources have been reported which included insecticidal, antibacterial, antifungal and cytotoxic properties (Ang *et al.*, 2001; El Sayed *et al.*, 2001; Cafieri *et al.*, 1995; Cafieri *et al.*, 1996; Nakamura *et al.*, 1984; Tsukamoto *et al.*, 1999; Edrada *et al.*, 1996). Three isomalabaricane triterpenes have been isolated from the marine sponge *Rhabdastrella globostellata* and the results showed that these compounds were toxic to human colon tumor (Tasdemir *et al.*, 2002). Manzamines isolated from *Xestopongia ashmorica* were found to be toxic to a mouse lymphoma cell line (Edrada *et al.*, 1996). This compound is reported to also found in other

genera of marine sponges, including Pellina, Pachypellina, Xestospongia, Ircinia and Amphimedon (Ang et al., 2000). However, none of these compounds are originated and developed in Indonesia which is known to be rich of marine biodiversity.

This study is aimed to study cytotoxicity of an alkaloid compound isolated from Indonesian marine invertebrates (sponge) collection number MD-02; the marine sponges which were collected from Bunaken, Indonesia. The outcome of this research is to obtain lead compounds with potential biological activities against cancer cell lines originated from Indonesia.

Materials and Methods

- Extraction and Isolation

Isolation of the alkaloid compound was conducted based on bioassay-guided isolation developed by Alam et al., 2005. The small pieces of sponges were extracted with acetone. After removal the solvent by evaporation, the residue was partitioned with chloroform. Separation of the active compound (designated as compound A) is performed on chloroform extract using Vacuum Liquid Chromatography followed by TLC-preparative.

- Cytotoxicity Testing

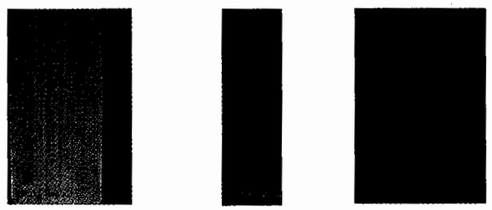
Testing for toxicity were conducted in 96 well plates employing two types of cancer cell lines (HeLa and Raji) growing in RPMI 1640 media and one normal cell (Vero cells) growing in M199 media. The media were supplemented with streptomycin-penicillin 1% and fungizon 1%. Microwell (96 well plate) were seeded at a density of 4 x 10^4 cells/well. After 24 h of plating and growth, cells were incubated with compound A. Each 96 well plate had a negative control (no compound) and blank (no cell). Cell were incubated with the compounds at the concentration of 25, 12,5, 6,25, 3,125, 1,56 and 0,78 µg/ml for 24 h, at which time MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide thiazolyl blue) was added (10 µL of 5 mg/mL stock) for 4 h. 100 µL stopping reagent containing SDS wasadded to dissolve the formazan and the absorbance was read on plate reader at λ= 540 nm.

- Determination of Cell Morphology and Cell Death

The cell morphology was examined using phase contrast microscope. Agarose gel electrophoresis was used to detect the DNA ladder pattern of the treated cells.

Results and Discussion

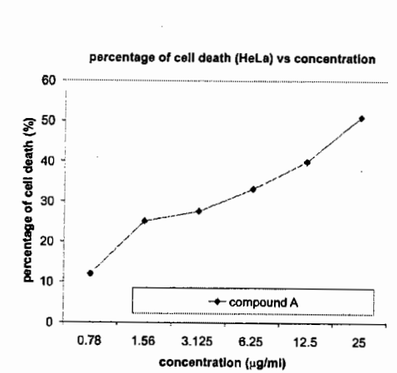
Samples collected from Bunaken Bay were extracted using acetone employing maceration technique. Acetone was chosen with consideration that it was able to extract non-polar as well as polar compounds with minimal sea salt contamination. Following maceration with acetone, partition was conducted using chloroform followed by methanol. Chloroform fraction was separated by Vacuum liquid chromatography. From the nine fractions obtained by VLC, fraction 7 was shown to contain the desired alkaloid compound, similar with that as observed by Alam et al., 2005. This compound was further purified by TLC-preparative. The alkaloid compound was obtained with hRf of 67 in TLC system with n-hexane: EtOAc:Amonia = 1:1:0.03 v/v as the mobile phase and SiO2 gel F254 as the stationary phase. This compound showed positive result with Dragendorf spot detection, showing that the isolate was alkaloid (figure 1a) and this spot was strongly fluorescence under UV366 lamp (figure 1b) and absorb UV light at UV254 (figure 1c)



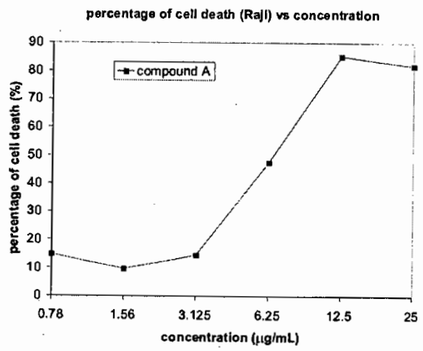
(a) (b) (c)

Figure 1. TLC profiles of compound A detected using (a) Dragendorf (b) UV 254 (c) UV 366. CHCl3 = Chloroform fraction, Cmp A = isolated alkaloid. Stationary phase: SiO2 gel F254, mobile phase: n-hexane:EtOAc:Amonia= 1:1:0.03 v/v

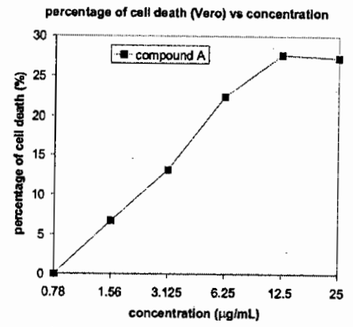
In-vitro cytotoxicity study was undertaken to demonstrate the effects of the isolated alkaloid on different classes of human cancer and normal cells. The purpose of the study was to determine whether this compound has a selective cytotoxic effect against cancer cells. MTT based cytotoxic assay was carried out using two cancer (Raji and HeLa cells) and one normal cells (Vero). This compound exhibited significant growth inhibition of the two cancer cells that were tested and at the concentrations of 25 µg/mL, the cell survival of HeLa cells was less than 50%. This compound was found to be more toxic to Raji cells which showed cell survival of less than 50% at concentration of 12.5 mg/mL. Cytotoxicity towards Hela and Raji cells is in a dose dependent manner (Figure 2a and 2b). LC_{50} value of the isolated alkaloid towards HeLa and Raji cells were 22.57 and 6.37 µg/mL, respectively.



(a)



(b)



(c)

Figure 2. Cytotoxic effect of isolated alkaloid on (a) HeLa cells (b) Raji cells (c) Vero cells

In addition to their effects on cancer cells, the compound was used against Vero, normal cell line. This was conducted in order to determine whether its effect was selective. The results indicated that unlike cancer cells, in all tested concentrations, the compound was found to cause less than 50% of cell death. This compound appeared to have low cytotoxicity towards Vero cells (Fig.2c) with LC_{50} of 100.36 µg/mL.

Exposure of HeLa and Raji cells to the isolated compound resulted in morphological changes characteristic of apoptotic death (Figure 3). This data was supported by the DNA ladder pattern of the treated cells (at the concentration of 6.25 µg/mL) which showed cleavage of chromosomal DNA into fragments (Figure 4). Apoptosis is a highly conserved phenomenon that plays an important role in the regulation of cellular activities. Apoptosis is characterized by certain distinct morphological (cell shrinkage, membrane blebbing, pyknosis, chromatin margination, cytoplasm contraction and condensation) and biochemical (DNA fragmentation into distinct ladders or degradation of apoptotic markers such as PARP and nuclear lamins) features. Apoptosis and the genes that control it has a profound effect on the malignant phenotype (Tang and Porter, 1997). These results provide a new insight that this

bioactive compound is promising and could be further developed for anticancer agent.

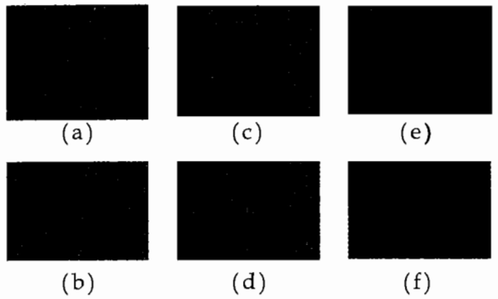


Figure 3. Morphology changes of HeLa, Raji and Vero cells under microscope. (a) Control Hela cells, (b) Hela cells treated with compound A, (c) Control Raji cells, (d) Raji cells treated with compound A, (e) Control Vero cells, (f) Vero cells treated with compound A.

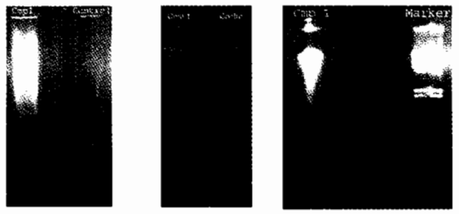


Figure 4. DNA ladder pattern of treated Raji with control untreated cells (a) and treated Hela with control untreated cells (b), treated HeLa with marker 100 bp (c)

Conclusion

An alkaloid compound was isolated from chloroform fractions of sponges MD-02. This compound was toxic to Raji than HeLa cells with LC_{50} of 22.67 and 6.37 µg/mL, respectively. Cells treated with this compound showed cleavage of chromosomal DNA into fragments, suggesting the possibility of apoptotic cell death. This compound was less toxic to normal Vero cells showing its selectivity with LC_{50} of 100.36 µg/mL.

Acknowledgement

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